J. Glob. Innov. Agric. Soc. Sci., 2015, 3(2-3): 43-49.
ISSN (Online): 2311-3839; ISSN (Print): 2312-5225
DOI: 10.17957/JGIASS/3.2-3.708
http://www.jgiass.com

LEAF MORPHOLOGY, SCLEROPHYLLY AND LEAF WATER RELATIONS OF SOME FIELD-GROWN OLIVE (*OLEA EUROPAEA* L.) CULTIVARS IN TUNISIA

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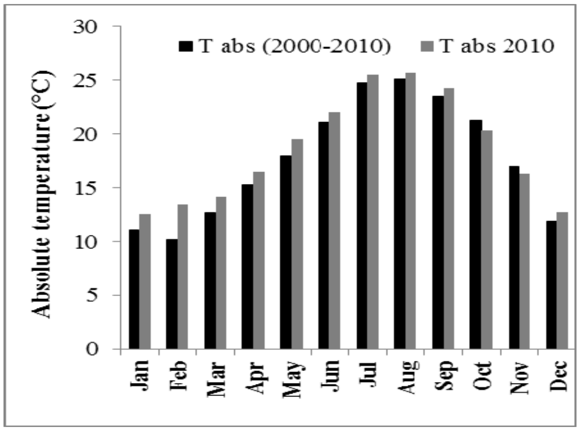
With the increasing effect of global warming, olive growing is facing very severe conditions. In Tunisia, the rainfall deficit reached in some areas more than 50% of the normal amount and the temperature rose considerably. The present study was conducted in order to understand the behavior of the olive trees to these changes. We found considerable morpho-structural leaf–differences among the cultivars. Based on the study of leaf structure, 'Dahbia' leaves showed the highest value of ratio (palisade/spongy parenchyma). So, this cultivar enhanced its sclerophylly by building parenchyma tissues. Also, 'Besbessi' showed the lowest total lamina with high value of leaf stomatal resistance. In fact, to cope with the environmental stress conditions and water scarcity, olive cultivars used different strategy to overcome the surrounding circumstances. 'Fougi' showed the lowest relative water content when 'Lucques' exhibited good protection against water loss through the lowest values of water saturation deficit associated to the low values of stomatal resistance and leaf water potential. Concerning the chlorophyll fluorescence, the maximum quantum yield as the ratio Fv/Fm revealed that, for all studied cultivars, the functional integrity of photosystem II was not affected. The results obtained indicated good plasticity of olive leaves to cope with stress. The leaf mechanisms employed are very various (morpho-structural and eco-physiological) and differ from cultivar to another.
Keywords: water stress, leaf tissues thickness, leaf anatomy, leaf water status, fluorescence.

INTRODUCTION

In Tunisia, olive cultivation plays an important role in agriculture (it has the second largest olive forest in the world with an area of 1.8 million hectares, covering almost 80% of tree areas) and in economic activities. Thus, the development of a strategy for olive agro-ecological adaptation requires an understanding of relationships between genotype and environment, phenotypic plasticity and dynamic adaptation of varieties to environmental impacts, especially when drought and water scarcity are prevailing. Olive trees bear drought conditions for a very long time and may continue to grow even in low rainfall (Fereres, 2004). Olive plants adapt well to low water conditions (Chaves *et al*., 2002). Bussoti *et al.* (2002) have observed that this plant may overcome the harsh environmental conditions with an increase in sclerophylly. Because the leaf is the most flexible organ in its response to environmental conditions (Nevo *et al*., 2000). Stressed leaves are characterized by smaller mesophyll cells, less intercellular space (Mediavilla *et al*., 2001) and by a greater density of stomata (Chartzoulakis *et al*., 2000). For this species, the stomatal closure is a mechanism used by olive trees to prevent loss of water on days of high atmospheric demand (Fernández, 2013). The stomatal resistance depends on leaf water status and tends to increase with the installation of stress. There are some factors which are major reasons for stomatal transpiration includes density, opening level of stomata and structure. Irradiance, CO_2 concentration and humidity are also included in factors responsible for stomatal transpiration (Royer, 2001). The control of excessive transpiration during midday hours and the maintenance of high relative water content with decreasing the leaf water potential may contribute to the stress tolerance (Choudhury *et al*., 2014). Maintaining a high potential in the plant appeared to be linked to an optimization of the water absorption by the roots. Therefore, a plant which prevented dehydration retains a low leaf water potential when subjected to water deficit. It was also able to maintain adequate tissue hydration, allowing normal metabolic function, either by reducing losses or increasing the absorption of water (Radhouane, 2007). The present study was conducted in order to understand the adaptation of olive trees to stress on the basis of leaf morphological and anatomical variations. We compared the olive cultivars with some foreigners in order to observe structural and morphological changes which decrease loss of water and to precise leaf-level mechanisms which make olive trees able to deal with environmental stress conditions.



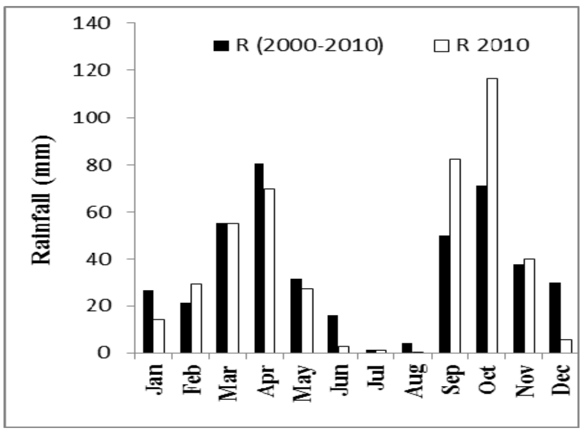


Figure 1. Monthly air absolute temperatures and rainfall (T abs = mean for the period 2000–2010 and T abs 2010 = mean for the year 2010) and rainfall (R = mean for the period 2000–2010 and R 2010 = mean for the year 2010) at the experimental station of Chott Mariem.

MATERIALS AND METHODS

Study site: Experimentation was performed in a sandy-soil in Chott Mariem (35°54’N; 10°33’E) (Sousse, Tunisia) at 250m above sea level. The climatic conditions are naturally Mediterranean with severe drought and high temperatures in summer (Figure 1). There is negligible rainfall in summer but mostly falls are in autumn season. Figure 1 shows the variation of mean values of rainfall and temperature in 2010. January and February are the coldest while July and August are the warmest months. In autumn season, rainfall was relatively high (reaching up to 116 mm in October). In the three months of summer (June, July and August), rainfall was reached out zero, ensuring the growth of fruits in drought conditions.

Plant material, leaf anatomy study and tissue measurements: Unirrigated, nineteen cultivars of open field-grown with 28-year age olive trees were studied. The local cultivars were Chemchali, Beldi, Chetoui, Besbessi, Marsaline, Roumi, Chamlali, Meski, Gerboui, Oueslati, Fougi, Sayali, Tounsi and R’khami. The foreigner cultivars were Lucques, Picholine, Dahbia, Ascolana and Manzanilla. Leaf material was collected four times a year, every three months as once a season in 2010 and all functional properties and tissue measurements were studied on 27 (9 per tree) healthy, sun exposed, mature leaves per cultivar. The breadth of spongy parenchyma and palisade, lower and upper epidermis measured in cross-sections of leaf, for that purpose fresh material was brought for examination through microscope. Middle sections of leaves were taken to avoid differential thickness.

Abaxial surface of each leaf was coated once with polish for stomatal impressions; then with great care polish was peeled off and positioned on slide of microscope. The stomatal and trichomes densities (trichomes mm^{-2}) were determined on the measured surface of fresh fully developed leaves of three olive trees from each cultivar. Peltate trichomes were detached from the inferior surface of the leaves, than by using adhesive tape before the measurement of the stomatal density. The observations were recorded after every three months with the help of Windias software and Leitz Dialux 22 EB microscope, with the enlargement 250 times.

Morphology, sclerophylly and leaf water relations: At noontime, matured leaves were collected from each of three sunny trees, once each month throughout 2010. The succeeding factors were studied: leaf area of one leaf (LA (cm^2), measured using a microscope type Leitz Dialux 22 EB and the Windias software (Windows), Fresh Mass (FM; g), fresh mass at full turgor (TM (g), measured after immersion of leaf petioles in demineralized water for 48 h in the dark at 4 °C) and Dry Mass (DM (g), measured after drying at 70°C to a constant weight). The Specific Leaf Area (SLA) was determined as the ratio of LA to DM (cm^2 g^{-1}). The leaf tissue density (D) calculated as (DM/FM) × 1000 and expressed in g kg^{-1} (DM/FM; g kg^{-1}) were calculated according to Groom and Lamont (1999). Several indices of leaf water status were also calculated: Relative Water Content (RWC= ((FM – DM)/(TM – DM))*100; %), succulence (S = (FM – DM)/LA; mg H_2O cm^{-2}), Water Saturation Deficit (WSD = ((TM – FM)/(TM – DM))*100; %) and Water Content at Saturation (WCS = (TM - FM)/DM; g H_2O g^{-1}DM).

Resistance of leaf stomatal was calculated in the mid of each month with the help of porometer (LI-1600; LI-COR Inc., Lincoln, NE, USA). The dominant part of the leaf hemisphere took place in the cup at head-unit which was situated at right angles to sun. The observations were taken between 10 and 11 am on the abaxial surface of well-exposed leaves. Stomatal resistance was measured on two different leaves per tree, with three replications.

The water leaf potential (MPa) was performed in the mid of each month throughout 2010 at midday by Scholander Pressure chamber Model SKPM 1400 (Skye Instruments, Powys, UK). The measurements were taken on two mature leaves per plant with three replications. The leaf was enclosed in a plastic bag immediately after cutting from the tree and the water potential determination for leaf executed within a minute.

Leaf chlorophyll fluorescence: The fluorescence of Chlorophyll was determined in the mid of each month throughout 2010 (in the same days of the two precedent parameters) between 11 am and 1 pm with the help of fluorometer leaf chamber (LI-6400-40; Li-Cor, Inc.). Chlorophyll maximum fluorescence (Fm) and basal fluorescence (Fo) were observed in extended leaves. The maximum quantum yield of PSII photochemistry (Fv/Fm) was calculated as (Fm-Fo)/Fm (Oxborough, 2004). The measurements were carried out on two leaves per tree covered by domestic clip-holders for 50 minutes before the calculation (three trees per cultivar were used).

Statistics: For all parameters and in all tables, the values are given as mean value ± standard deviation (SD). The comparison between the behaviors of the 19 cultivars was made using Analysis of Variance (ANOVA). Means were determined by Duncan's test with significance level ($P<0.05$). All statistical procedures were performed using SPSS 16 statistical software.

RESULTS AND DISCUSSION

Leaf anatomy and tissues study: The leaves of olive trees may show some adaptive traits in their constitutional internal structure of different cell layers. The variations in thickness of lamina between cultivars were due to difference in proportionality of leaf mesophyll composition. In our study, the lamina was thick in 'Tounsi' and 'R'khami' but thin in 'Besbessi' and 'Marsaline'. The leaves' lamina of 'Besbessi' was the thinnest (500,77 µm) (Table 1). Chartzoulakis *et al*., (2000) reported that the mesophyll breadth decreases under stress condition which shows cell size reduction due to water stress, it is likely to be mechanism of drought adaptation. The ratio palisade to spongy parenchyma was high in 'Chemlali' (0,55), showing a dense cell arrangement and high surface area of mesophyll to unit area of leaf which helps in uptake of CO_2 and photosynthesis (Chartzoulakis *et al.* 2000). 'R'khami' had the highest value of spongy parenchyma but the lowest leaf tissue ratio (Table 1). Leaves of 'Meski' had the thinnest palisade parenchyma and the lowest leaf thickness ratio. However, 'Dahbia' had the lowest spongy parenchyma (268 µm) and the highest leaf tissue ratio (Palisade parenchyma/Spongy parenchyma) (0,64) and 'Tounsi' presented the highest thickness of total lamina and spongy parenchyma (Table 1). Highly dependent of spongy parenchyma to total lamina (µm) was also found and could be expressed as follows: Spongy parenchyma = 0,673 total lamina – 30,637 (Figure 2 A) with a correlation coefficient r^2 = 75%.These variations of leaf anatomy characteristics may explicate the various stratagems of olive cultivars under stress situations. A thicker upper epidermis (including upper cuticle) and a thicker palisade parenchyma in some cultivars such as 'Chemlali' may increase their endurance and development under stress circumstances with high fortification for internal tissues (Chartzoulakis *et al.,* 2000, Mediavilla *et al.,* 2001).

Morphology, sclerophylly and leaf water status: The lowest ratio (dry weight/fresh weight) of leaves was 51,9% for 'Chemchali' while it didn't exceed 69% for 'R'khami'. Concerning the foreign cultivars, the highest ratio was recorded for 'Dahbia' (64,86%) (Table 2). Results relative to the weight of the leaves (g) showed high correlation between the dry and fresh weight (g) (Figure 2 B). From this correlation, we deduced that about 70% of leaves were water. The relationship between these weights was expressed by the following equation: Dry weight = 0,661 fresh weight – 0,011.

Table 1. Leaf tissue thickness (µm) and leaf tissue ratio (Palisade parenchyma/Spongy parenchyma) of 19 olive cultivars field-grown in Chott Mariem (Tunisia)

Cultivars	Total lamina (µm)	Upper epiderms[a] (µm)	Lower epiderms[a] (µm)	Upper palisade parenchyma (µm)	Spongy parenchyma (µm)	Palisade/spongy
Chemchali	556,40±1,38cd	28,90±0,29ghi	18,22±0,06bc	152,60±1,34ef	306,66±0,47bcde	0,49±0,08fg
Beldi	526,70±7,34a	23,77±0,09abc	19,04±0,04d	142,10±3,83cd	293,72±4,02bc	0,48±0,16efg
Chetoui	580,77±12,48fgh	27,50±1 fgh	18,55±0,13bcd	166,40±0,80ik	321,20±12,8ef	0,52±0,40ghi
Besbessi	500,77±5,78	24,10±0,35abcd	20,47±0,39e	128,80±3,23a	273,31±3,04a	0,47±0,11def
Marsaline	516,70±4,12a	27,90±0,64gh	18,11±0,11bc	127,84±2,48a	302,79±2,23bcd	0,42±0,11abc
Roumi	551,57±11,89bcd	25,40±0,50cde	21,96±0,42f	132,59±5,65ab	321,57±6,11ef	0,41±0,20ab
Chemlali	575,77±1,43efg	29,23±0,67hi	17,21±0,07a	171,84±2,65k	309,44±2,94cde	0,55±0,24i
Meski	539,10±6,22abc	24,40±0,26bcd	17,26±0,17a	127,44±0,84a	322,95±5,91ef	0,39±0,09a
Gerboui	517,63±2,5a	28,37±0,24gh	21,66±0,70f	136,39±0,90bc	291,13±3,88b	0,46±0,16def
Oueslati	559,73±3,31cde	29,40±0,35hi	19,08±0,33d	171,90±1,34k	309,72±0,61cde	0,49±0,15efg
Fougi	560,87±2,88def	27,47±0,57fgh	23,12±0,12g	162,17±3,70ghi	302,04±4,19bcd	0,53±0,31hi
Sayali	580,67±4,06ghi	30,73±0,58i	18,69±0,26cd	165,46±1,29hik	322,71±5,18ef	0,51±0,19gh
Tounsi	611,87±11,9k	26,93±0,35efg	21,59±0,26f	158,95±1,30fgh	358,34±11,78hi	0,44±0,25bcd
R'khami	600,73±6,73ik	28,10±0,55gh	19,04±0,04d	144,28±0,58d	363,29±5,73i	0,39±0,08a
Lucques	530,20±2,13ab	25,87±1,09def	17,94±0,06abc	140,24±1,08cd	302,10±0,40bcd	0,46±0,06def
Picholine	559,93±2,14hik	27,13±0,80efg	23,22±0,16g	156,45±1,60efg	345,05±3,19gh	0,45±0,15cde
Dahbia	535,03±5,92ab	27,03±0,87efg	17,78±0,22ab	151,47±2,86e	268,27±5,96a	0,64±0,32
Ascolana	572,23±2,92efg	22,43±0,93a	20,30±0,10e	169,42±1,08ik	314,99±3,21ef	0,53±0,15hi
Manzanilla	591,03±5,21ghi	23,03±0,30ab	19,37±0,16d	172,07±0,37k	331,50±5,71fg	0,52±0,17ghi

Values represent the mean (±SE) of three replications. [a] Values also include the cuticle layer. Means within each column followed by different letters are significantly different ($P<0.05$, Duncan test).

The maintaining of cell turgor is generally regarded as one of the means by which the negative influence of liquid stress on the metabolic processes of plant can be reduced. For the two cultivars from the north, 'Besbessi' showed the highest leaf surface area (23,86 mm^2) whereas the lowest value (9,23 mm^2) was recorded for Meski (Table 2). The total biomass production of plants and the growth of the aerial parts are significantly affected by water (Zgallaï *et al.*, 2007). The small leaves growth may help in water losses reduction in 'Meski' and balancing the adverse anatomical structures. In comparison, the growth of large leaves in 'Besbessi' may be liable to dehydration. Concerning the specific leaf area, it is frequently reduced under drought situations and its deviations may be caused by the change in the thickness of leaf (Veneklaas *et al.*, 2002). This may explain the lowest values that were recorded for the cultivars from the north in our work.

Concerning trichomes density, the foreign cultivar 'Lucques' showed 257 trichomes mm^{-2}, 'Oueslati' and 'Marsaline' had 151 trichomes mm^{-2} (Table 2). Leaf water status may be improved through leaf hairs by collecting and holding apparent water, support to their ultimate absorption in the mesophyll. The highest values of stomatal density corresponded to the two local cultivars, from south of Tunisia, 'Chemchali' (548 stomata mm^{-2}) and 'Tounsi' (487 stomata mm^{-2}). The lowest values were attributed to 'Besbessi' (258 stomata mm^{-2}) and 'Marsaline' (278 stomata mm^{-2}) from north (Table 2). Our results demonstrated that the lowest stomatal densities were in local olive cultivars from north of Tunisia compared with those from the south. Concerning the leaf density tissue, 'Sayali' (519 g kg^{-1}) and 'Lucques' (534 g kg^{-1}) showed low values of D (Table 3). 'Manzanilla' had the highest density of foliar tissue (690 g kg^{-1}) and the lowest RWC (30%) (Table 3). These outcomes were verified by the functional studies showing a thinner upper epiderms for this cultivar and a dense aggregation of cells with greater density of trichomes. Due to the dehydration and high resistance to physical damage, it is greater possibilities that tissue with high density might survive more under severe drought conditions (Mediavilla *et al*. 2001). The relatively high values of D in 'Chemchali', 'Chetoui', 'Marsaline', 'Fougi' and 'Chemlali' cultivars corroborated the anatomical studies that exposed a thinner spongy parenchyma for these varieties and it is may be relevant to less volume of inter-cellular spaces. Niinemets (2001) reported that leaves containing lower D are less stable rather than the higher D, it is vital reason for long life time. Considering the RWC values (Table 3), all cultivars extended to an adequate water stress level. A decline in RWC is stated to be close related with tolerance of drought (Wan *et al*., 1998). 'Lucques' had low RWC and high WCS, indicating high water loss. Based on the value of WSD, 'Lucques' used a tolerance desiccation process that simplified the high assimilatory rates during the onset deficit of water (Gulías *et al.* 2002). 'Fougi' revealed low WCS and WSD values (Table 3). It may have a more aptitude to endure arid environments.

Stomatal resistance and minimum leaf water potential: However the information is precious that obtained from the water contents for the drought measuring events, but the important factors are osmolarity and tissue's water potential for specific plant tolerance-desiccation (Radhouane, 2007). During the experimental period, the stomatal resistance varied considerably with cultivars. The lowest values were 0,99 s cm^{-1}, 1,08 s cm^{-1} and 1,13 s cm^{-1} for, respectively, 'Lucques', 'Chemchali' and 'Marsaline' (Figure 3 A). We registered the highest values for the two local cultivars 'Beldi' and 'Tounsi' and for the introduced 'Manzanilla',

Table 2. Ratio (Dry weight/fresh weight) (%), leaf area (mm^2), specific leaf area (SLA) (cm^{2}g^{-1}), trichomes density (trichomes mm^{-2}) and stomatal density (stomata mm^{-2}) of 19 olive cultivars field-grown in Chott Mariem

Cultivars	Dry Weight/Fresh Weight (%)	Leaf surface (mm^2)	SLA (cm^2 g^{-1})	Trichomes density (trichomes mm^{-2})	Stomatal density (stomata mm^{-2})
Chemchali	51,90±9,10a	11,70±0,85ab	41,42±1,37cd	163,80±4,10ab	548,73±16,38ag
Beldi	56,98±8,30ab	14,24±0,67ab	37,02±1,46abc	221,13±6,49bcd	343,98±14,64bc
Chetoui	58,96±29,27abc	12,72±0,56abc	37,39±0,25abc	196,56±14,19abcd	389,03±21,67cd
Besbessi	54,59±18,34a	23,86±1,02c	47,66±4,60de	171,99±12,67abd	257,99±13,94ad
Marsaline	60,13±13,93abc	12,34±1,85ab	30,38± 0,10ab	151,52±20,48a	278,46±20,48abe
Roumi	60.31±27.38ab	16.00±4.31b	176±3.00abc	200.66±24.91abcd	389.03±17.85cdg
Chemlali	64.38±9.34abc	17.55±1.24b	168.33±0.33ab	171.99±24.57ab	397.22±16.38cd
Meski	60.66±19.31abc	9.23±1.73a	186.33±5.67bcd	208.85±12.29abcd	454.55±12.29de
Gerboui	61.38±22.34bc	14.68±3.12ab	165.33±8.84ab	237.51±22.80cd	470.93±21.67de
Oueslati	57.27±6.54bc	13.78±4.00ab	175.33±4.67abc	151.52±17.85a	421.79±67.41cde
Fougi	59.38±10.89abc	13.68±0.07ab	184.00±12.00abcd	176.09±4.10abc	397.22±8.19cd
Sayali	53.42±7.78abc	16.99±3.37b	170.67±11.33abc	204.75±39.06abcd	450.45±47.23de
Tounsi	58.26±43.90ab	14.94±0.95ab	196.67±19.33cd	188.37±28.67abc	487.31±16.38eg
R'khami	69.00±44.15ab	11.44±1.32ab	183.33±8.41abcd	184.28±24.57abc	454.55±25.57de
Lucques	57.46±14.39a	12.00±3.21ab	167±7.39ab	257.99±13.39d	429.98±18.93de
Picholine	60.91±23.71ab	14.32±1.78ab	181.33±15.62abcd	188.37±22.80abc	421.79±21.67cde
Dahbia	64.86±18.38ab	14.22±2.38ab	177±10.10abc	184.28±11.29abc	393.12±14.39cd
Ascolana	56.43±22.39abc	12.39±4.28ab	158±12.39a	171.99±9.39ab	442.26±10.29de
Manzanilla	60.24±87.87c	16.68±0.98b	170±7.55abc	204.75±22.80abcd	405.41±24.57cd

Values represent the mean (±SE) of three replications. Means within each column followed by different letters are significantly different (P<0.05, Duncan test).

Table 3. Relative water content (RWC) (%), density of the leaf tissue (D) (g kg^{-1}), succulence (S) (mg H_2O cm^{-2}), water content at saturation (WCS) (g H_2O g^{-1}DM) and water saturation deficit (WSD) (%) of 19 olive cultivars field-grown in Chott Mariem

Cultivars	RWC (%)	D (g kg^{-1})	S (mg H_2O cm^{-2})	WCS (g H_2O g^{-1}DM)	WSD (%)
Chemchali	39,26±2,12abcd	613,78±7,45abc	22,39±0,66bc	1,45±0,15b	10,74±2,12abcd
Beldi	32,83±2,99abc	575,78±21,53ab	20,39± 0,02abc	1,56±0,15bc	17,17±2,99bcd
Chetoui	32,96±1,27abc	589,59±5,08ab	18,91±1,05abc	1,54±0,06bc	17,04±1,27bcd
Besbessi	46,51±3,65cd	545,91±25,47a	17,45± 0,12abc	0,98±0,41ab	13,49±3,65ab
Marsaline	36,10±3,53abcd	601,30± 0,31abc	23,04±4,07bc	1,25±0,13ab	14,90±3,53abcd
Roumi	33,15±3,68abc	574,61±18,46ab	18,47±3,32abc	1,56±0,12bc	16,85±3,68bcd
Chemlali	43,78±3,94bcd	609,07±9,24abc	17,85±1,17abc	0,83±0,25ab	16,22±3,94abc
Meski	37,01±2,77abcd	603,09±9,56abc	17,80±0,80abc	1,30±0,25ab	15,99±2,77abcd
Gerboui	34,46±0,70abcd	648,63± 0,21bc	16,96±3,16abc	1,05±0,02ab	15,54±0,70abcd
Oueslati	39,19±6,42abcd	643,82±17,51bc	23,07±5,44bc	0,88±0,37ab	15,81±6,42abcd
Fougi	60,61±4,16	606,64±27,42abc	24,27±0,12c	0,43±0,20a	9,39±4,17
Sayali	44,38±0,78bcd	519,01±9,10a	18,55±1,66abc	0,84±0,06ab	15,62±0,78abc
Tounsi	37,22±2,70abcd	572,71±6,54ab	21,37±1,37abc	1,30±0,13ab	16,78±2,70abcd
R'khami	35,63±8,53abcd	593,77±12,39ab	20,99±2,68abc	1,46±0,17b	14,37±8,54abcd
Lucques	27,47±3,14a	534,23±2,45a	15,53± 0,01ab	1,74±0,07c	7,53±3,14
Picholine	40,39±5,03abcd	582,63±3,69ab	19,91±1,3abc	1,12±0,17ab	14,61±5,03abcd
Dahbia	48,08±6,20d	564,33±43,90ab	20,10±0,01abc	0,84±0,18ab	15,92±6,20a
Ascolana	30,47±2,01ab	602,43±43,89abc	14,57±3,68a	1,53±0,22bc	16,53±2,02cd
Manzanilla	30,03±4,31ab	690,03±22,41c	22,17± 0,02abc	1,02±0,27ab	16,97±4,31cd

Values represent the mean (±SE) of three replications. Means within each column followed by different letters are significantly different (P<0.05, Duncan test).

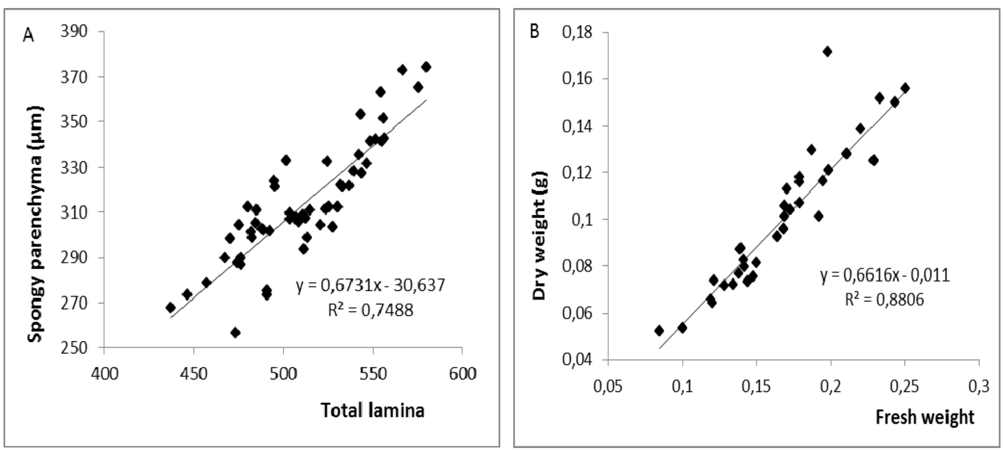


Figure 2. Relationship between spongy parenchyma and total lamina (µm) (A) and between dry weight and fresh weight of leaves (g) (B) determined for 19 cultivars of *Olea europaea* L.

respectively, 2,75 s cm^{-1}, 2,56 s cm^{-1} and 2,67 s cm^{-1} (Figure 3 A). According to Soleimani *et al.*, (2010), the olive cultivars 'Frantoio' and 'Zard', with low stomatal resistance, were more tolerant to stress. The increase of stomatal resistance is caused by the closing of stomata and opposed the distribution of water vapor and gases (Denden and Lemeur, 2000).

Fernández *et al.* (1997) and Moriana *et al.* (2003) observed that under high temperature conditions, olive plants can reduce an excessive water loss by closing their stomata. Chartzoulakis *et al.* (1999) showed that stomatal resistance increased with the degree of water deficit in the olive trees.

Furthermore, Bongi and Palliotti (1994), in mature olives 'Frantoio', and Fernández and Moreno (1999), in developed trees of 'Manzanilla', noticed that stomatal closing activity seems to be facilitating by single translocated upcoming from stressed root developing in areas where low water potential reduced water uptake. On the other hand, scarcity of water affected the most physiological parameters such as the leaf water potential. In our conditions, the values of the minimum water potential varied from -2,23 MPa for 'Roumi' to -3,22 MPa for 'Chemlali' (Figure 3 B). The foreign cultivars 'Lucques', 'Picholine' and 'Manzanilla' showed similar values (-2,9 MPa) (Figure 3 B). The local cultivars showed a wide range of minimum leaf water potential variations. Based to our results, in order to adapt to its environment and to overcome the state of stress, olive occured either by closing their stomata therefore increased the values of stomatal resistance as the varieties 'Besbessi', 'Tounsi', 'Fougi' and 'Meski' or by decreasing the leaf water potential as the cultivars 'Chemchali', 'Chemlali', 'Oueslati', 'Dahbia', 'R'khami', 'Picholine' and 'Lucques', or through the two phenomena together like with 'Beldi' and 'Manzanilla'.

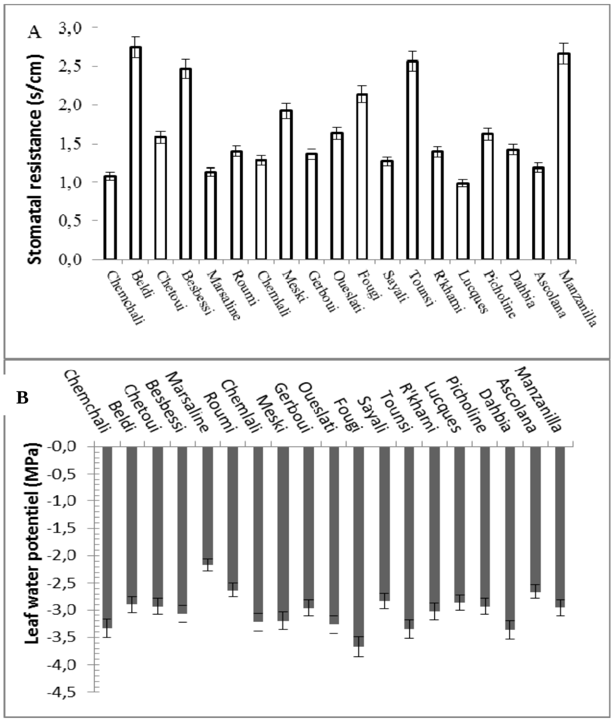


Figure 3. Stomatal resistance (s cm^{-1}) (A) and leaf water potential (MPa) at midday (B) of 19 cultivars of *Olea europaea* L. field-grown in Chott Mariem (Tunisia). Vertical lines indicate the standard deviations.

Leaf Chlorophyll Fluorescence: In order to judge the effect of abiotic stress on the operating effectiveness of the photosynthesis process performed by the leaves, measurements of chlorophyll fluorescence were held. Fluorescence of Chlorophyll can be adopted for early stress indication. Photosynthesis and fluorescence are inter-related so that recommended for globally vegetation monitoring status. Under stress, plant tissues raise heat production to disperse surplus energy (Angelopoulos et *al*., 1996). Concerning our study, the original level (Fo) of chlorophyll fluorescence showed the highest values for the cultivars 'Chemlali', 'Ascolana', 'Roumi' and 'Manzanilla' (Figure 4 A). The Fv/Fm showed the lowest level with 'Sayali' (Figure 4 B). Variances in the ratio Fv/Fm were not significant between cultivars, and the lowest value (0,64) was recorded for 'Chemlali' (Figure 4 B). Deviations in Fv/Fm showed changing in efficiency of photo-chemical for PSII, whereas the values found for the majority of plants (near 0,8) were in limits the healthy plants values. A reduction of this ratio in 'Chemlali' plants was due to a reduction in Fm and revealed increasing in energy dissipation due a destruction of the photosynthetic apparatus. Fluorescence observation for environmental parameters may be the result of degradation of chlorophyll and/or deficiency of synthesis, together with thylakoid membrane integrity decline. In some circumstances, light-dependent inactivation of PSII reaction centers was related with decrease in both Fv/Fm and Fm, with increment in initial yield of fluorescence Fo (Long and Humphries, 1994). Also identified that increased temperature leads to

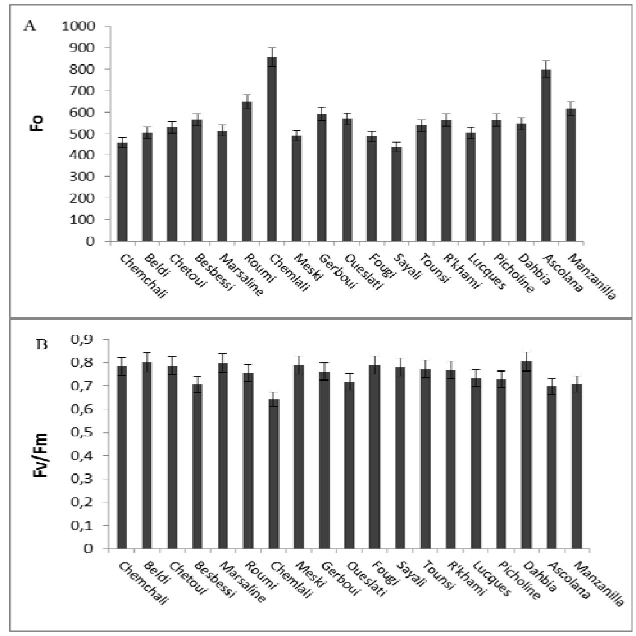


Figure 4. Chlorophyll fluorescence measured for 19 olive cultivars field-grown in Chott Mariem (Tunisia): (A) basal fluorescence (Fo); (B) ratio (Fv/Fm). Vertical lines indicate standard deviation.

thermal damaging, later on, rise in Fo (Ludlow and Björkman, 1984). In comparison to absolute yields of Fo, the ratio of Fv/Fm was independent of above artifacts and indication for photo inhibition of photosynthesis and as sensitive indicator of cell health.

CONCLUSION

This study allowed us to depict nineteen olive cultivars that showed different level of adaptability with the environmental conditions of the east coastal center in Tunisia. Based on their eco-physiological attitudes, it was possible to classify the cultivars in three different groups. The first contains 'Beldi' and 'Manzanilla' that are the most suitable cultivars with high stomatal resistance and low leaf water potential. The second is reserved for cultivars with low stomatal resistance and high leaf water potential. These cultivars are struggling to withstand the conditions of the region; here we find 'Marsaline', 'Roumi' and 'Chetoui'. The last group is formed with all the rest of the cultivars that are moderately adapted. The variability of morphology, sclerophylly and water relations indices of leaf become as visible and direct expression of physiological changes into the plant. These changes take place to help the olive overcoming the stress, by increasing the RWC, D and the ratio (dry weight/fresh weight) in 'Fougi', 'Dahbia', 'Chemchali' and 'R'khami' or by increasing the density of trichomes such in 'Lucques' or reducing the stomata density as in 'Besbessi' or the leaf area such in 'Meski'. In conclusion, significant differences were found between the studied olive cultivars considering their aptitude to avoid, limit or support water stress. Obtained data represent additional support to the hypothesis that the adaptation mechanisms of olive trees to their environment are various.

Our results may be useful for olive plantation programs in central Tunisia.

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